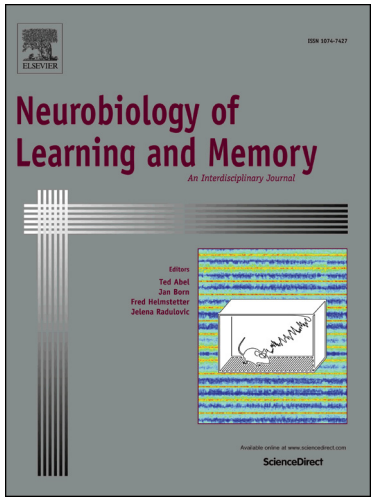
DOI:
10.1016/j.nlm.2016.07.018

Document Version
Peer reviewed version



Contextual Fear Conditioning Induces Differential Alternative Splicing

Shane G. Poplawski, Lucia Peixoto, Giulia S. Porcari, Mathieu E. Wimmer, Anna G. McNally, Keiko Mizuno, K. Peter Giese, Snehajyoti Chatterjee, John N. Koberstein, Davide Risso, Terence P. Speed, Ted Abel

PII: S1074-7427(16)30118-6
DOI: http://dx.doi.org/10.1016/j.nlm.2016.07.018
Reference: YNLME 6493

To appear in: *Neurobiology of Learning and Memory*

Received Date: 24 March 2016
Revised Date: 16 July 2016
Accepted Date: 19 July 2016

Please cite this article as: Poplawski, S.G., Peixoto, L., Porcari, G.S., Wimmer, M.E., McNally, A.G., Mizuno, K., Peter Giese, K., Chatterjee, S., Koberstein, J.N., Risso, D., Speed, T.P., Abel, T., Contextual Fear Conditioning Induces Differential Alternative Splicing, *Neurobiology of Learning and Memory* (2016), doi: http://dx.doi.org/10.1016/j.nlm.2016.07.018

Contextual Fear Conditioning Induces Differential Alternative Splicing

Abbreviated Title: Alternative splicing after contextual learning

Shane G. Poplawski[1†], Lucia Peixoto[2,3†], Giulia S. Porcari[2], Mathieu E. Wimmer[4], Anna G. McNally[1], Keiko Mizuno[5], K. Peter Giese[5], Snehajyoti Chatterjee[2], John N. Koberstein[3], Davide Risso[6], Terence P. Speed[7,8,9] and Ted Abel[1,2*]

[1]Pharmacology Graduate Group, University of Pennsylvania, Philadelphia, PA, USA; [2]Department of Biology, University of Pennsylvania, Philadelphia, PA, USA; [3]Elson S. Floyd College of Medicine, Washington State University, Spokane, WA, USA; [4]Department of Psychiatry, University of Pennsylvania, Philadelphia, PA, USA; [5]Centre for the Cellular Basis of Behaviour, King's College London, London, UK [6]Division of Biostatistics, School of Public Health, University of California, Berkeley, California, USA; [7]Department of Statistics, University of California, Berkeley, California, USA; [8]Bioinformatics Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia; [9]Department of Mathematics and Statistics, The University of Melbourne, Victoria, Austraila

* Corresponding Author
† These authors made equivalent contributions to the paper

Ted Abel
Department of Biology, University of Pennsylvania, Philadelphia, PA 19104-6018, USA
Phone: +1 215 746 1122, E-Mail: abele@sas.upenn.edu

Current Addresses:
Shane G. Poplawski – Sr. Research Scientist, Ibis Biosciences, Carlsbad, CA, USA
Lucia Peixoto – Assistant Professor, Washington State University Spokane, Spokane, WA, USA

Number of pages: 16
Number of figures/tables: 3
Words in Abstract: 153
Words in Introduction: 553
Words in Discussion: 893
Total number of words: 3000

Conflict of interest: None

Keywords: transcription, alternative splicing, RNA-seq, fear conditioning, Homer1

Acknowledgments: This research was supported by NIH R01MH087463 to T.A.

Abstract

The process of memory consolidation requires transcription and translation to form long-term memories. Significant effort has been dedicated to understanding changes in hippocampal gene expression after contextual fear conditioning. However, alternative splicing by differential transcript regulation during this time period has received less attention. Here, we use RNA-seq to determine exon-level changes in expression after contextual fear conditioning and retrieval. Our work reveals that a short variant of Homer1, *Ania-3*, is regulated by contextual fear conditioning. The ribosome biogenesis regulator *Las1l*, small nucleolar RNA *Snord14e*, and the RNA-binding protein *Rbm3* also change specific transcript usage after fear conditioning. The changes in *Ania-3* and *Las1l* are specific to either the new context or the context-shock association, while the changes in *Rbm3* occur after context or shock only. Our analysis revealed novel transcript regulation of previously undetected changes after learning, revealing the importance of high throughput sequencing approaches in the study of gene expression changes after learning.

1. Introduction

Contextual fear conditioning requires two waves of transcription and protein synthesis in the hippocampus to form long-term memory (Bourtchouladze et al., 1998; Igaz et al., 2002). Our lab and others have focused on discovering the genes regulated during these transcriptional waves using both candidate gene and genome-wide approaches. Our microarray-based studies have indicated that the first wave of transcription induces the largest change in gene expression 30 minutes after contextual learning (Peixoto et al., 2015b). However, gene regulation is a complex process that has multiple layers of control. Levels of particular mRNA isoforms can be regulated by alternative start sites, differential splicing including exon skipping and intron retention, and alternative poly(A) site selection (Leff et al., 1986; Raj and Blencowe, 2015). Alternative splicing can lead to distinct protein function and interactions (Ellis et al., 2012) or regulate mRNA localization (Ehlers et al., 1998; Jaskolski et al., 2004; Papandrikopoulou et al., 1989), and thus is expected to be particularly important in neurons, which need to traffic mRNA to their long cellular processes.

Most previous research studying genome-wide gene expression in the hippocampus after contextual learning has relied on microarray technology (Barnes et al., 2012; Cavallaro et al., 2002; Keeley et al., 2006; Klur et al., 2009; Levenson et al., 2004; Mei et al., 2005; Peixoto et al., 2015b). Although microarrays are a reliable tool to measure changes in gene expression, they are unable to distinguish exon-level effects that are indicative of alternative splicing. RNA-seq provides numerous advantages over microarrays (Peixoto et al., 2015a), including the ability to study exon-level changes in gene expression. Isoform-specific gene expression changes are known to occur after fear conditioning, including upregulation of *Bdnf IV*, but not other *Bdnf* isoforms (Lubin et al., 2008; Mizuno et al., 2012), and *Homer1a*, but not *Homer1c* (Mahan et al., 2012) in response to strong, three shock training protocols. The different *Bdnf* isoforms have distinct transcription start sites, while the expression of *Homer1* isoforms is controlled by the splicing regulator SRp20 (Wang et al., 2014), which is upregulated after

learning (Antunes-Martins et al., 2007). These examples indicate that gene regulation after learning is more complex than gene-level differences and can be highly selective for particular isoforms of a gene.

Therefore, we used RNA-seq to study differential alternative splicing 30 minutes after contextual fear conditioning and 30 minutes after memory retrieval. Applying Remove Unwanted Variation (RUV), a recently designed normalization algorithm (Peixoto et al., 2015a; Risso et al., 2014), to our data, we discovered 171 bins, corresponding to either an entire exon or any portion of a gene, across 138 genes that showed differential expression after learning independent of changes at the gene-level. After memory retrieval 450 differentially expressed bins corresponding to 311 unique genes were discovered. These bins include retained introns, unique start/end sites, or small RNA not yet spliced out of the polyadenylated mRNA. The differences include *Snord14e*, a small nucleolar RNA, which our lab has previously shown to be regulated at this time point (Peixoto et al., 2015b). Sno-RNAs, which are commonly found within introns of genes, regulate RNA processing and have been implicated in memory consolidation (Rogelj et al., 2003). In addition, *Ania-3*, an alternative short form of Homer1 that has not previously been linked to learning, ribosome biogenesis regulator *Las1l*, and the RNA-binding protein *Rbm3* were also regulated by contextual fear conditioning. These findings demonstrate that alternative splicing is regulated by contextual learning on a genome-wide scale and also identify novel candidate isoforms that may be pertinent to memory consolidation.

2. Materials and Methods

Subjects

C57Bl/6J mice were maintained under standard conditions with food and water available *ad libitum*. Adult male mice 2 months of age were kept on a 12-hr light/12-hr dark cycle with lights on at 7AM. All behavioral and biochemical experiments were performed during the light cycle with training starting at 10AM (ZT3). All animal experiments were approved by the Institutional

Animal Care and Use Committee of the University of Pennsylvania and were consistent with National Institutes of Health guidelines.

Behavior

Contextual fear conditioning was performed as previously described (Hawk et al., 2012; Vecsey et al., 2007) with handling for 3 days prior to conditioning. Briefly, the conditioning protocol entailed a single 2-sec, 1.5mA footshock terminating at 2.5 minutes after placement of the mouse in the chamber. Mice were left in the chamber for an additional 30 seconds and then returned to their homecage. One mouse per behavioral group (homecage, fear conditioned) was trained per day over 10 days to reduce unwanted variation caused by training and sacrifice times. One mouse was also tested the next day to ensure proper freezing levels (Peixoto et al., 2015b).

RNA isolation

Hippocampi were dissected either from homecage mice or 30 minutes after training and placed into RNAlater (Qiagen Valencia, CA) and frozen on dry ice. Tissue was homogenized using a TissueLyser system and RNA was extracted using the RNAeasy Microarray Tissue kit (Qiagen) according to the manufacturer's instructions. Samples were DNase treated using the RNase-Free DNase kit (Qiagen) off-column by incubating 5ul DNase and 35ul Buffer RDD for 25min at RT with each sample. Samples were then ethanol precipitated and resuspended in water.

RNA-seq Library Preparation and Sequencing

2µg of RNA from n=5 homecage and fear conditioned mice was used in the TruSeq RNA Sample Prep Kit (Illumina San Diego, CA) according to the manufacturer's instructions with polyA selection. Completed libraries were size-selected on an agarose gel to remove any high basepair fragments, quantified by qPCR (KAPA Biosystems Boston, MA), and submitted to the

PGFI sequencing core at the University of Pennsylvania. An Illumina HiSeq 2000 sequenced the libraries in paired-end 100bp reads. 3 libraries were sequenced per lane on an Illumina HiSeq 2000, resulting in an average of 67,011,105 reads per sample in the homecage mice and 62,115,805 reads per sample after fear conditioning. Reads had good unique concordance (86.9% in homecage, 85.5% after fear conditioning) and mapping (90.7% of unique concordant reads in homecage and 93.1% after fear conditioning). RNA-seq data is available through GEO (GSE63412) (Peixoto et al., 2015a).

Data Analysis

Sequencing reads were aligned to the mouse mm9 genome using GSNAP (Wu and Nacu, 2010) (http://share.gene.com/gmap). An exon-level count table was produced by counting reads into unique, non-overlapping "bins" using Ensembl gene models and HTSeq (Anders et al., 2012) (http://www-huber.embl.de/users/anders/HTSeq/doc/overview.html). A "bin" can either be any part of a gene or an entire exon depending on the uniqueness of the region. Bin counts were normalized using upper-quartile scaling implemented in edgeR (Robinson et al., 2010) followed by RUVs, which corrects for unwanted variation using replicate/negative control samples (Risso et al., 2014). Additionally, we used 8897 bins residing in 625 genes identified as unchanged from a previous microarray experiment as negative controls for RUV under the assumption that these bins are also not changing (Peixoto et al., 2015a; Peixoto et al., 2015b). We discovered that four factors of unwanted variation (k=4) need to be adjusted for to resolve the differences caused by contextual fear, which was chosen using the method described by Peixoto et al. (Peixoto et al., 2015a). Differential splicing analysis was performed with the limma Bioconductor package, using the voom and diffSplice functions (Law et al., 2014; Ritchie et al., 2015). Functional annotation was performed through DAVID (Huang da et al., 2009a, b) (http://david.abcc.ncifcrf.gov/). The annotation was limited to the following sources: GO

Biological process, GO Molecular Function, KEGG pathways, and SwissProt and Protein Information Resource keywords and an EASE score restriction of 0.1.

qPCR analysis

RNA was isolated from a separate cohort of fear conditioned, immediate shock, or context only mice following the same behavioral paradigms described above. Immediate shock consisted of placing the mouse in the context with the footshock on and immediate removal, while context involved placing the mouse in the context for the same time as contextual conditioning with no shock. RNA was converted to cDNA using the RETROscript kit (Ambion) according to the manufacturer's instructions. cDNA reactions were diluted to 200ul and 2.25ul was combined with 0.25ul 5µM primer mix and 2.5ul SYBR Select Master Mix (Life Technologies Carlsbad, CA) and run on a Viia7 Real Time PCR system. The ΔΔCt method was used for analysis (Poplawski et al., 2014), with all primers showing >90% efficiency. The primers used were: Ania1F-AGTGGCTGGTTTTCTTGGACT, Ania1R-GGGAGGTGGATTGGTGACAA, Homer1Bin21F-CTGGAGTCCACTGCCAATGT, Homer1Bin21R-CTCTGCTTCCTCCTGGTACG, Las1lBin15F-TCAAAGTCAGAGGGGTCGGA, Las1Bin15R- AGACTTCGCTCTTGCTGCTT, Las1lBin17F-TGCTGGAGAAACACAGGCAT, Las1lBin17R- ACATTGTACACGTGGGGAAAGA, Rbm3Bin2F-ACCTGAGTTTTGGAGGCTGG, Rbm3Bin2R- ACAACAGCGGACACCATAGG, Rbm3Bin7F-GGTGGCTATGACCGCTACTC, Rbm3Bin7R- TTTTGTGTGCATGCCCCATC, Rbm3Bin22F-TGCCCCTGGCAGACATAGAG, Rbm3Bin22R- GTCTGCCACTTTCTTCGTTCTTT. The comparison between three groups (homecage, immediate shock, context only) was analyzed using an ANOVA. The effect of bin ($F(7, 160) = 11.90$), condition ($F(2, 160) = 7.835$) and interaction ($F(14, 160) = 3.719$) were all significant, and Tukey tests were used to determine the significance of each bin.

3. Results

RNA-seq has the advantage of distinguishing exon-level reads that are difficult to identify by any other method, and therefore it is an ideal technique to study alternative splicing. We used RNA-seq to study gene expression in the hippocampus 30 minutes after contextual fear conditioning, a time point our lab has previously determined to show robust expression changes after fear conditioning (Peixoto et al., 2015b). We used GSNAP (Wu and Nacu, 2010) to align reads to the mm9 mouse genome and HTSeq (Anders et al., 2015) to count reads into bins (Anders et al., 2012) using Ensembl gene models. Bins are separated based on overlap of Ensembl gene models, with any unique section of a transcriptional unit split into a separate bins. Therefore, a bin can represent either a whole exon or any other unique portion of the gene model. Thus, differential start sites, 3' ends, or retained introns can be observed as unique bins if they are part of the Ensembl database. So as not to bias ourselves using gene models, we considered every bin as a potential site for alternative regulation. RUVs normalization performed as described (Risso et al., 2014), adjusting for four factors of unwanted variation (which can include biological and technical noise), was found to control for fear conditioning as the primary effector of variation between samples. Bioconductor package limma was then used to determine differential bin usage independent of gene-level changes (Ritchie et al., 2015). We identified 171 bins across 138 genes that displayed differential usage (FDR<0.05) after contextual fear conditioning (**Table 1**). 129 of these bins were upregulated and 42 were downregulated, consistent with the general increase in gene expression after fear conditioning (Peixoto et al., 2015b). We performed functional classification of genes showing at least 1 bin-specific change after fear conditioning. The SwissProt and Protein Information Resource keywords "phosphoprotein" and "alternative splicing" were enriched in our data set, indicating that our exon-level analysis discovers alternative splicing as expected. Clusters corresponding to protein catabolic processes and nucleotide binding were also enriched. The same analysis was performed on samples 30 minutes after memory retrieval (testing). In this analysis, we found 450 bins corresponding to 311 unique genes (Table 2). This list of genes contains 70 of the 138

genes observed to change after fear conditioning, highlighting the overlap between memory consolidation and retrieval (Peixoto et al., 2015b).

Upregulated bins during memory consolidation included *Snord14e*, which reside in the introns of the *Hspa8* gene. We have recently validated *Snord14e* upregulation after detecting differences by microarray (Peixoto et al., 2015b). We also discovered that a poorly studied short isoform of *Homer1* known as *Ania-3 (Ensembl Homer1-005)* (Bottai et al., 2002) is upregulated after contextual fear conditioning. *Homer1a* has previously been shown to be upregulated by fear conditioning (Mahan et al., 2012), but *Ania-3* has not been studied. To validate our results, we performed qPCR in a separate cohort of mice, comparing the bins observed to change to a bin of the same gene that was unchanged. *Ania-3* was found to be upregulated independently of the entire *Homer1* gene (**Figure 1a**). Ribosome biogenesis protein *Las1l* exhibited bin-specific downregulation in response to contextual fear conditioning and this was also confirmed by qPCR (**Figure 1b**). RNA-binding protein *Rbm3*, which our lab has shown to change in the hippocampus after sleep deprivation (Vecsey et al., 2012), displays complex regulation with both upregulated and downregulated bins after learning. Both the upregulated and downregulated bins were confirmed by qPCR in a separate cohort of animals (**Figure 1c**). In all cases, the bin predicted to change was significantly regulated while a control bin in the same gene was unchanged.

To test whether these changes are specific to the association of context and shock, a separate cohort of animals were either immediately shocked or exposed to the context with no footshock. Expression of *Ania-3* shows a change in response to context only, but not after immediate shock (**Figure 2**). This is not surprising given the overlap of gene expression between fear conditioning and spatial training (Keeley et al., 2006; Poplawski et al., 2014). An increase in *Ania-3* may represent splicing changes in response to a novel environment. *Las1l* displays a non-significant trend toward a reduction in the context only, but not immediate shock (**Figure 2**). *Las1l* bin 15 may represent a splicing change that occurs only with a context-shock

association. *Rbm3* bin 2 expression shows changes in both the immediate shock and context only conditions, (**Figure 2**), suggesting that this alternative splicing occurs with minimal perturbation and may not reflect a learning event. *Rbm3* bin 22 showed only a trend toward a decrease in both cases. Therefore, changes in *Rbm3* bin 2 may represent any activity within the hippocampus, while *Rbm3* bin 22 could be specific to context-shock associations. These results indicate that alternative splicing can occur in response to a variety of factors and may be a specific marker in the hippocampus of recent behavioral stimuli.

4. Discussion

In this study, we provide the first evidence of genome-wide regulation of alternative splicing after learning in the hippocampus. Using bin counts produced by HTSeq and the limma Bioconductor package, we compared bins representing a unique piece of a gene against expression of that entire gene to create a list of bin-level changes. We were able to detect significant gene expression changes at 171 bins occurring in response to contextual fear conditioning at 138 genes. The exact number of potential splicing sites is not known in neurons, and splicing studies have identified as low as 3110 splicing events in neurons (Zhang et al., 2014) or as high as 92-94% of all genes (Wang et al., 2008). We suspect that 138 genes showing changes is only a small fraction of the potential change. It is unclear at this time why memory retrieval shows a larger set of changes than memory consolidation. This study used whole hippocampus, so only a small fraction of all cells in the sample are being activated by learning. However, our RUV analysis removes unwanted variation including that from nonresponsive cells, so we believe that the changes observed are due to activated neurons.

Although individual examples of alternative splicing have been observed during memory consolidation (Lubin et al., 2008; Mahan et al., 2012; Rozic et al., 2011), no studies have explored this phenomenon genome-wide. We also identified candidate genes displaying alternative regulation that may be important for learning. As previously reported (Peixoto et al.,

2015b), we confirmed that *Snord14e*, which exists within an intron of *Hspa8*, is regulated by fear conditioning. It is unclear why *Snord14e* increases in polyadenylated RNA, but it could be due increases in intron retention during transcription or splicing and polyadenylation of a *Snord14e* precursor. We also implicate the selective alternative splicing of *Homer1* isoform *Ania-3,* RNA-binding protein *Rbm3* and ribosome biogenesis regulator *Las1l* in learning for the first time. These results emphasize the importance of using genome-wide binning techniques to identify subtle changes in splicing following fear conditioning, which would be overlooked with standard RNA-seq analysis.

It is interesting that we observed different results for *Rbm3* alternative splicing in the context only and immediate shock controls. At the gene-level, gene expression changes after contextual and spatial learning is known to overlap (Poplawski et al., 2014). Previous work from our lab has highlighted similar gene expression between fear conditioning and context-only exposure in the hippocampus, but not the amygdala (Keeley et al., 2006). Thus, we anticipated similar results between our fear conditioning results and the context only control, as was the case with all splicing events tested. This confirms our previous findings that exposure to a context is sufficient to elicit similar gene expression changes that occur when context is paired with shock.

However, we found that *Rbm3* had a unique response to the components of fear conditioning, with an immediate shock being able to alter alternative splicing of bin 2 of this gene. Immediate shock does not provide the subject enough time to form a contextual representation of the space, and therefore is generally thought not to cause expression changes in the hippocampus (Huff et al., 2006). Thus, the change after immediate shock in *Rbm3* may suggest that splicing of *Rbm3* bin 2 in the hippocampus is altered by many brain stimuli. In contrast, *Rbm3* bin 22, *Ania-3* and *Las1l* may be specific to exposure to a novel context or context-shock association. Thus, splicing changes in *Rbm3* bin 2 may not be involved in forming long-term contextual fear memories, while splicing in *Ania-3* and *Las1l* may have a role in

encoding these types of memories. We hypothesize that this may be an instance of a broader phenomenon in neuronal plasticity, where certain splicing events are regulated by any neuronal activity while others only respond to specific stimuli.

In the present study, we did not determine the type of splicing that is occurring at each of these bins, which will be the subject of future analyses. Each bin observed could be the result of many types of regulation, including exon skipping, intron retention, or alternative start/stop sites. *Ania-3* has previously been reported as an alternative isoform of Homer1 that responds like an immediate early gene (Bottai et al., 2002), and the change we detect here corresponds to the unique *Ania-3* exon. Whether *Ania-3* splicing is regulated by SRp20, as is the case for *Homer1a* (Wang et al., 2014), is a subject for future investigation. *Las1l* bin 15 appears to be a retained intron, but whether this is part of the canonical *Las1l* mRNA or part of a different isoform is unknown. It is also possible that this could be a small RNA that is spliced out of the final polyadenylated mRNA. *Rbm3* bin 2 is one of several potential transcriptional termination sites of *Rbm3* while bin 22 could either be an early termination site or a retained intron. Further study will be required to determine the exact identity and function of the isoforms that are regulated by contextual fear conditioning.

The mechanism that drives this alternative splicing is not studied within these experiments, although transcription of certain splicing proteins such as SRp20 is known to change after fear conditioning (Antunes-Martins et al., 2007). Recent studies have also highlighted the importance of Rbfox1 in splicing and mRNA regulation in neurons (Lee et al., 2016). Our data indicates regulation of a specific isoform of splicing factor *Sfpq*. However, it is unclear whether these transcriptional changes would be translated into protein and affect splicing by 30 minutes after training. The mechanism by which alternative regulation of transcripts is controlled during memory consolidation is an important question for future studies. It is possible that changes in epigenetic modifications are regulating this selective transcript usage (Zhou et al., 2014), including H3K36me3 and H4K20me1 (Luco et al., 2010; Zhu et al.,

2013). It would be interesting to observe whether the differential bins discovered in this study show differential histone modifications as well. We hope our findings and this unique analysis method drive further study into the mechanisms of isoform-specific changes in gene expression during memory consolidation.

Figure Captions

Table 1. List of bins showing differential expression after fear conditioning. Each differential bin contains the chromosome, start position, and end position for easy reference.

Table 2. List of bins showing differential expression 30 minutes after memory retrieval. Each differential bin contains the chromosome, start position, and end position for easy reference.

Figure 1. Bin-specific regulation of *Homer1* (*Ania-3*), *Las1l*, and *Rbm3*. A) (left) diffSplice result showing the predicted significant bin changes of the *Homer1* gene in red on a $\log_2$ scale. Bins 16-18 indicate the *Ania-3* isoform. (right) qPCR validation of the change in Bin18 in an independent cohort of mice. Bin 21 expression was compared as a control. B) (left) diffSplice result showing the predicted significant bin changes of the *Las1l* gene in red on a $\log_2$ scale. (right) qPCR validation of the change in Bin 15 in an independent cohort of mice. Expression of Bin 17 was used as a control. C) (left) diffSplice result showing the predicted significant bin changes of the *Rbm3* gene in red on a $\log_2$ scale. (right) qPCR validation of the changes in Bin 2 and Bin 22 an independent cohort of mice. Expression of Bin 7 was used as a control. HC=homecage, FC=fear conditioned. *denotes a p-value of <0.05.

Figure 2. Alternative splicing changes variably in response to either component of fear conditioning alone. The same primers used in Figure 1 were used to test gene expression after context only (n=8) or immediate shock controls (n=8) or homecage animals (n=7) and analyzed by ANOVA. *Ania-3 and Las1l* changes in response to the context only control and *Rbm3* changes with all manipulations. This may suggest *Ania-3* and *Las1l* are markers of contextual novelty while *Rbm3* responds to many stimuli. No control bins change with either

context-only or immediate shock controls. HC=homecage, CTX=context only, SH=shock only.

*denotes significance below an alpha of 0.05.

References

Anders, S., Pyl, P.T., and Huber, W. (2015). HTSeq — A Python framework to work with high-throughput sequencing data. Bioinformatics *31(2)*, 166-169.

Anders, S., Reyes, A., and Huber, W. (2012). Detecting differential usage of exons from RNA-seq data. Genome research *22*, 2008-2017.

Antunes-Martins, A., Mizuno, K., Irvine, E.E., Lepicard, E.M., and Giese, K.P. (2007). Sex-dependent up-regulation of two splicing factors, Psf and Srp20, during hippocampal memory formation. Learn Mem *14*, 693-702.

Barnes, P., Kirtley, A., and Thomas, K.L. (2012). Quantitatively and qualitatively different cellular processes are engaged in CA1 during the consolidation and reconsolidation of contextual fear memory. Hippocampus *22*, 149-171.

Bottai, D., Guzowski, J.F., Schwarz, M.K., Kang, S.H., Xiao, B., Lanahan, A., Worley, P.F., and Seeburg, P.H. (2002). Synaptic activity-induced conversion of intronic to exonic sequence in Homer 1 immediate early gene expression. J Neurosci *22*, 167-175.

Bourtchouladze, R., Abel, T., Berman, N., Gordon, R., Lapidus, K., and Kandel, E.R. (1998). Different training procedures recruit either one or two critical periods for contextual memory consolidation, each of which requires protein synthesis and PKA. Learn Mem *5*, 365-374.

Cavallaro, S., D'Agata, V., Manickam, P., Dufour, F., and Alkon, D.L. (2002). Memory-specific temporal profiles of gene expression in the hippocampus. Proceedings of the National Academy of Sciences of the United States of America *99*, 16279-16284.

Ehlers, M.D., Fung, E.T., O'Brien, R.J., and Huganir, R.L. (1998). Splice variant-specific interaction of the NMDA receptor subunit NR1 with neuronal intermediate filaments. J Neurosci *18*, 720-730.

Ellis, J.D., Barrios-Rodiles, M., Colak, R., Irimia, M., Kim, T., Calarco, J.A., Wang, X., Pan, Q., O'Hanlon, D., Kim, P.M., *et al.* (2012). Tissue-specific alternative splicing remodels protein-protein interaction networks. Molecular cell *46*, 884-892.

Hawk, J.D., Bookout, A.L., Poplawski, S.G., Bridi, M., Rao, A.J., Sulewski, M.E., Kroener, B.T., Manglesdorf, D.J., and Abel, T. (2012). NR4A nuclear receptors support memory enhancement by histone deacetylase inhibitors. The Journal of clinical investigation *122*, 3593-3602.

Huang da, W., Sherman, B.T., and Lempicki, R.A. (2009a). Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic acids research *37*, 1-13.

Huang da, W., Sherman, B.T., and Lempicki, R.A. (2009b). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature protocols *4*, 44-57.

Huff, N.C., Frank, M., Wright-Hardesty, K., Sprunger, D., Matus-Amat, P., Higgins, E., and Rudy, J.W. (2006). Amygdala regulation of immediate-early gene expression in the hippocampus induced by contextual fear conditioning. J Neurosci *26*, 1616-1623.

Igaz, L.M., Vianna, M.R., Medina, J.H., and Izquierdo, I. (2002). Two time periods of hippocampal mRNA synthesis are required for memory consolidation of fear-motivated learning. J Neurosci *22*, 6781-6789.

Jaskolski, F., Coussen, F., Nagarajan, N., Normand, E., Rosenmund, C., and Mulle, C. (2004). Subunit composition and alternative splicing regulate membrane delivery of kainate receptors. J Neurosci *24*, 2506-2515.

Keeley, M.B., Wood, M.A., Isiegas, C., Stein, J., Hellman, K., Hannenhalli, S., and Abel, T. (2006). Differential transcriptional response to nonassociative and associative components of classical fear conditioning in the amygdala and hippocampus. Learn Mem *13*, 135-142.

Klur, S., Muller, C., Pereira de Vasconcelos, A., Ballard, T., Lopez, J., Galani, R., Certa, U., and Cassel, J.C. (2009). Hippocampal-dependent spatial memory functions might be lateralized in rats: An approach combining gene expression profiling and reversible inactivation. Hippocampus *19*, 800-816.

Law, C.W., Chen, Y., Shi, W., and Smyth, G.K. (2014). voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol *15*, R29.

Lee, J.A., Damianov, A., Lin, C.H., Fontes, M., Parikshak, N.N., Anderson, E.S., Geschwind, D.H., Black, D.L., and Martin, K.C. (2016). Cytoplasmic Rbfox1 Regulates the Expression of Synaptic and Autism-Related Genes. Neuron *89*, 113-128.

Leff, S.E., Rosenfeld, M.G., and Evans, R.M. (1986). Complex transcriptional units: diversity in gene expression by alternative RNA processing. Annual review of biochemistry *55*, 1091-1117.

Levenson, J.M., Choi, S., Lee, S.Y., Cao, Y.A., Ahn, H.J., Worley, K.C., Pizzi, M., Liou, H.C., and Sweatt, J.D. (2004). A bioinformatics analysis of memory consolidation reveals involvement of the transcription factor c-rel. J Neurosci *24*, 3933-3943.

Lubin, F.D., Roth, T.L., and Sweatt, J.D. (2008). Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. J Neurosci *28*, 10576-10586.

Luco, R.F., Pan, Q., Tominaga, K., Blencowe, B.J., Pereira-Smith, O.M., and Misteli, T. (2010). Regulation of alternative splicing by histone modifications. Science *327*, 996-1000.

Mahan, A.L., Mou, L., Shah, N., Hu, J.H., Worley, P.F., and Ressler, K.J. (2012). Epigenetic modulation of Homer1a transcription regulation in amygdala and hippocampus with pavlovian fear conditioning. J Neurosci *32*, 4651-4659.

Mei, B., Li, C., Dong, S., Jiang, C.H., Wang, H., and Hu, Y. (2005). Distinct gene expression profiles in hippocampus and amygdala after fear conditioning. Brain research bulletin *67*, 1-12.

Mizuno, K., Dempster, E., Mill, J., and Giese, K.P. (2012). Long-lasting regulation of hippocampal Bdnf gene transcription after contextual fear conditioning. Genes Brain Behav *11*, 651-659.

Papandrikopoulou, A., Doll, T., Tucker, R.P., Garner, C.C., and Matus, A. (1989). Embryonic MAP2 lacks the cross-linking sidearm sequences and dendritic targeting signal of adult MAP2. Nature *340*, 650-652.

Peixoto, L., Risso, D., Poplawski, S.G., Wimmer, M.E., Speed, T.P., Wood, M.A., and Abel, T. (2015a). How data analysis affects power, reproducibility and biological insight of RNA-seq studies in complex datasets. Nucleic acids research *43*, 7664-7674.

Peixoto, L.L., Wimmer, M.E., Poplawski, S.G., Tudor, J.C., Kenworthy, C.A., Liu, S., Mizuno, K., Garcia, B.A., Zhang, N.R., Giese, K.*, et al.* (2015b). Memory acquisition and retrieval impact different epigenetic processes that regulate gene expression. BMC Genomics *16 Suppl 5*, S5.

Poplawski, S.G., Schoch, H., Wimmer, M.E., Hawk, J.D., Walsh, J.L., Giese, K.P., and Abel, T. (2014). Object-location training elicits an overlapping but temporally distinct transcriptional profile from contextual fear conditioning. Neurobiology of learning and memory *116*, 90-95.

Raj, B., and Blencowe, B.J. (2015). Alternative Splicing in the Mammalian Nervous System: Recent Insights into Mechanisms and Functional Roles. Neuron *87*, 14-27.

Risso, D., Ngai, J., Speed, T.P., and Dudoit, S. (2014). Normalization of RNA-seq data using factor analysis of control genes or samples. Nature biotechnology.

Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W., and Smyth, G.K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic acids research *43*, e47.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics *26*, 139-140.

Rogelj, B., Hartmann, C.E., Yeo, C.H., Hunt, S.P., and Giese, K.P. (2003). Contextual fear conditioning regulates the expression of brain-specific small nucleolar RNAs in hippocampus. The European journal of neuroscience *18*, 3089-3096.

Rozic, G., Lupowitz, Z., Piontkewitz, Y., and Zisapel, N. (2011). Dynamic changes in neurexins' alternative splicing: role of Rho-associated protein kinases and relevance to memory formation. PloS one *6*, e18579.

Vecsey, C.G., Hawk, J.D., Lattal, K.M., Stein, J.M., Fabian, S.A., Attner, M.A., Cabrera, S.M., McDonough, C.B., Brindle, P.K., Abel, T.*, et al.* (2007). Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB:CBP-dependent transcriptional activation. J Neurosci *27*, 6128-6140.

Vecsey, C.G., Peixoto, L., Choi, J.H., Wimmer, M., Jaganath, D., Hernandez, P.J., Blackwell, J., Meda, K., Park, A.J., Hannenhalli, S.*, et al.* (2012). Genomic analysis of sleep deprivation reveals translational regulation in the hippocampus. Physiological genomics *44*, 981-991.

Wang, E.T., Sandberg, R., Luo, S., Khrebtukova, I., Zhang, L., Mayr, C., Kingsmore, S.F., Schroth, G.P., and Burge, C.B. (2008). Alternative isoform regulation in human tissue transcriptomes. Nature *456*, 470-476.

Wang, Q., Chikina, M.D., Pincas, H., and Sealfon, S.C. (2014). Homer1 alternative splicing is regulated by gonadotropin-releasing hormone and modulates gonadotropin gene expression. Mol Cell Biol *34*, 1747-1756.

Wu, T.D., and Nacu, S. (2010). Fast and SNP-tolerant detection of complex variants and splicing in short reads. Bioinformatics *26*, 873-881.

Zhang, Y., Chen, K., Sloan, S.A., Bennett, M.L., Scholze, A.R., O'Keeffe, S., Phatnani, H.P., Guarnieri, P., Caneda, C., Ruderisch, N.*, et al.* (2014). An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci *34*, 11929-11947.

Zhou, H.L., Luo, G., Wise, J.A., and Lou, H. (2014). Regulation of alternative splicing by local histone modifications: potential roles for RNA-guided mechanisms. Nucleic acids research *42*, 701-713.

Zhu, S., Wang, G., Liu, B., and Wang, Y. (2013). Modeling exon expression using histone modifications. PloS one *8*, e67448.

Figure 1

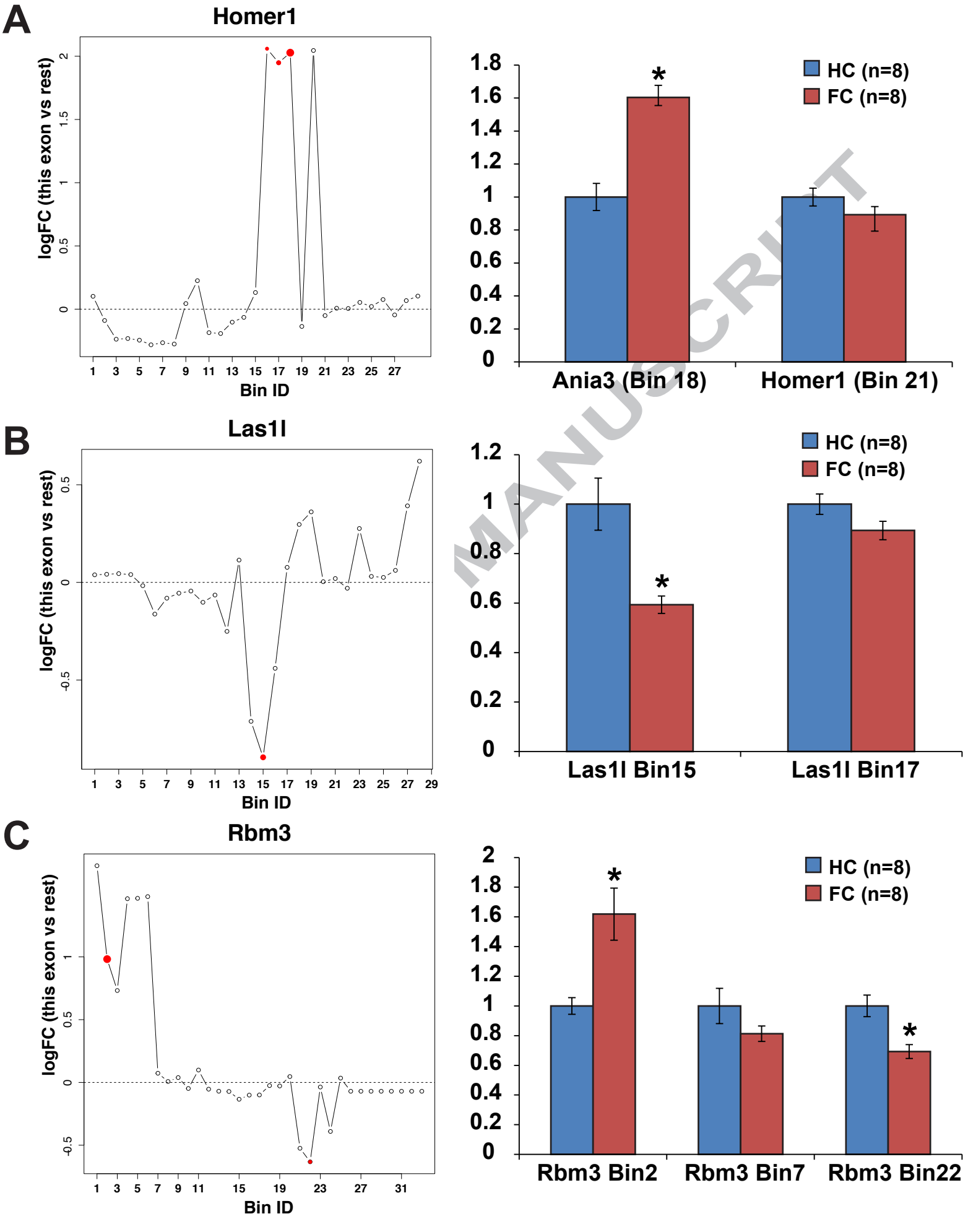


Figure 2

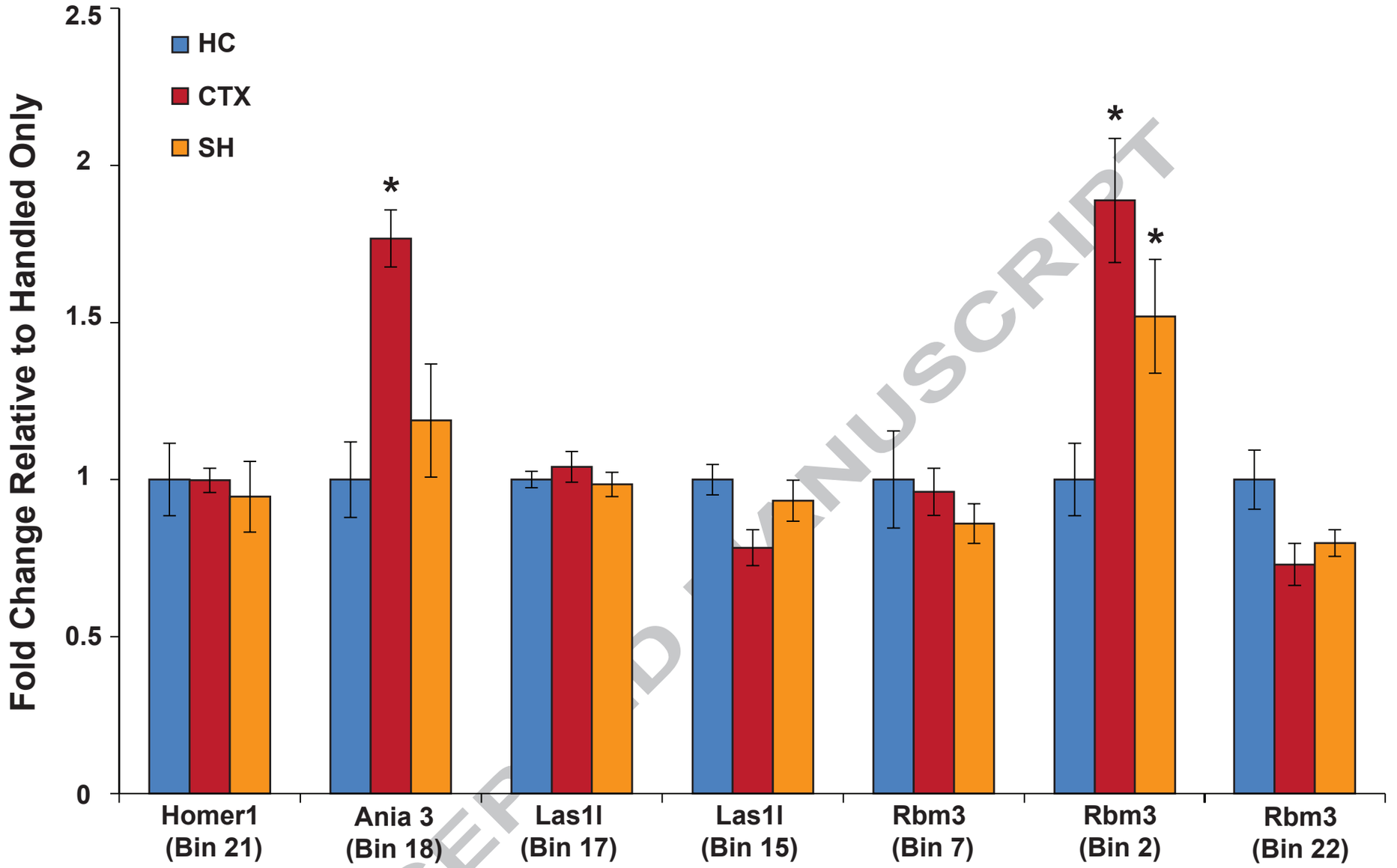


Table 1. Fear conditioned vs. homecage animals.

GeneID	Gene Name	Bin #	Chr	Start	Stop	Strand	logFC	t	P.Value	FDR
ENSMUSG00000007617	Homer1	018	chr13	94136356	94137083	+	2.0	14.1	1.4E-33	3.7E-28
ENSMUSG00000029657	Hsph1	012	chr5	150423259	150426039	-	1.1	10.3	2.4E-21	3.3E-16
ENSMUSG00000039801	2410089E03Rik	053	chr15	8201065	8202148	+	-1.1	-9.2	9.6E-19	8.6E-14
ENSMUSG00000031167	Rbm3	002	chrX	7716104	7717909	-	1.0	9.0	3.5E-17	2.3E-12
ENSMUSG00000007617	Homer1	017	chr13	94136233	94136355	+	1.9	8.4	3.5E-15	1.9E-10
ENSMUSG00000020431	Adcy1	024	chr11	7072883	7078509	+	-0.4	-8.4	5.5E-15	2.4E-10
ENSMUSG00000025372	Baiap2	027	chr11	119867673	119868096	+	0.5	7.7	3.5E-13	1.3E-08
ENSMUSG00000034083	C130022K22Rik	009	chr6	91835401	91838063	+	1.0	7.8	4.5E-13	1.5E-08
ENSMUSG00000008153	Clstn3	004	chr6	124383426	124383521	-	1.2	7.4	1.5E-12	4.5E-08
ENSMUSG00000043872	Zmym1	001	chr4	126724338	126724885	-	1.1	7.3	6.6E-12	1.8E-07
ENSMUSG00000005089	Slc1a2	036	chr2	102621901	102630941	+	-0.3	-6.7	8.4E-11	2.0E-06
ENSMUSG00000020287	Mpg	010	chr11	32130054	32131244	+	-0.6	-7.1	1.5E-10	3.3E-06
ENSMUSG00000024576	Csnk1a1	028	chr18	61745286	61746152	+	0.6	6.5	4.2E-10	8.5E-06
ENSMUSG00000063077	Kif1b	001	chr4	148550428	148552126	-	-0.2	-6.3	7.6E-10	1.5E-05
ENSMUSG00000035206	3110056O03Rik	016	chr10	80329406	80330144	+	0.4	6.5	9.0E-10	1.6E-05
ENSMUSG00000059495	Arhgef12	002	chr9	42771926	42776264	-	-0.3	-6.3	1.0E-09	1.7E-05
ENSMUSG00000022710	Usp7	020	chr16	8697013	8697568	-	0.8	6.2	1.4E-09	2.1E-05
ENSMUSG00000025372	Baiap2	026	chr11	119864352	119864399	+	0.6	6.3	1.6E-09	2.4E-05
ENSMUSG00000024576	Csnk1a1	027	chr18	61744853	61745285	+	0.6	6.2	2.3E-09	3.2E-05
ENSMUSG00000063077	Kif1b	002	chr4	148552127	148552971	-	-0.3	-6.0	4.0E-09	5.3E-05
ENSMUSG00000041879	Ipo9	036	chr1	137302594	137303043	-	-0.7	-5.9	5.5E-09	7.0E-05
ENSMUSG00000057421	Las1l	015	chrX	93143543	93144773	-	-0.9	-6.0	6.0E-09	7.3E-05
ENSMUSG00000034656	Cacna1a	068	chr8	87163334	87163334	+	1.4	5.9	7.4E-09	8.6E-05
ENSMUSG00000031878	Nae1	015	chr8	107042164	107043101	-	1.1	5.9	8.2E-09	9.1E-05
ENSMUSG00000023033	Scn8a	030	chr15	100869972	100876360	+	-0.2	-5.9	9.3E-09	1.0E-04
ENSMUSG00000075876+ENSMUSG00000064791+ ENSMUSG00000075924+ENSMUSG00000015656	Snord14c/Snord14e/Snord14d/Hspa8	038	chr9	40612831	40612920	+	1.4	5.9	9.7E-09	1.0E-04
ENSMUSG00000027523	Gnas	027	chr2	174155788	174155935	+	1.0	5.8	1.0E-08	1.0E-04
ENSMUSG00000071984	Fndc1	001	chr17	7931434	7932195	-	0.4	5.8	2.0E-08	1.9E-04
ENSMUSG00000038383	Pigu	004	chr2	155104386	155108131	-	0.5	5.8	2.6E-08	2.4E-04
ENSMUSG00000028053	Ash1l	002	chr3	88785155	88789712	+	0.4	5.7	3.0E-08	2.6E-04
ENSMUSG00000028826	Tmem57	002	chr4	134360480	134362431	-	-0.4	-5.7	3.9E-08	3.4E-04
ENSMUSG00000075876+ENSMUSG00000064791+ ENSMUSG00000075924+ENSMUSG00000015656	Snord14c/Snord14e/Snord14d/Hspa8	037	chr9	40612779	40612830	+	1.4	5.6	4.3E-08	3.6E-04

ENSMUSG00000024576	Csnk1a1	025	chr18	61742498	61744058	+	0.5	5.6	4.5E-08	3.7E-04
ENSMUSG00000023952	Gtpbp2	035	chr17	46303816	46303936	+	0.9	5.6	4.6E-08	3.7E-04
ENSMUSG00000007617	Homer1	016	chr13	94136198	94136232	+	2.1	5.6	5.4E-08	4.1E-04
ENSMUSG00000027429	Sec23b	030	chr2	144405140	144406851	+	0.9	5.5	6.4E-08	4.8E-04
ENSMUSG00000036052	Dnajb5	011	chr4	42963816	42965965	+	0.4	5.7	6.8E-08	4.9E-04
ENSMUSG00000013033	Lphn1	001	chr8	86424004	86424471	+	0.7	5.5	8.0E-08	5.6E-04
ENSMUSG00000035640	Dos	014	chr10	79598293	79598333	-	1.6	5.5	8.3E-08	5.7E-04
ENSMUSG00000028488	Sh3gl2	016	chr4	85033579	85035284	+	0.2	5.5	1.1E-07	7.5E-04
ENSMUSG00000027569	1600027N09Rik	010	chr2	180318228	180319110	+	0.4	5.5	1.5E-07	9.7E-04
ENSMUSG00000008153	Clstn3	005	chr6	124386790	124386835	-	1.4	5.3	2.1E-07	1.3E-03
ENSMUSG00000014873	Surf2	009	chr2	26773052	26774384	+	0.3	5.4	2.3E-07	1.5E-03
ENSMUSG00000063160+ENSMUSG00000003762	Numbl/Adck4	037	chr7	28047272	28049894	+	0.4	5.2	2.9E-07	1.8E-03
ENSMUSG00000024777	Ppp2r5b	006	chr19	6230276	6230385	-	0.5	5.3	3.1E-07	1.8E-03
ENSMUSG00000031167	Rbm3	022	chrX	7721600	7721698	-	-0.6	-5.2	3.1E-07	1.8E-03
ENSMUSG00000053580	Tanc2	043	chr11	105786047	105790613	+	-0.3	-5.2	3.3E-07	1.9E-03
ENSMUSG00000028161	Ppp3ca	030	chr3	136598842	136598864	+	0.7	5.2	3.5E-07	1.9E-03
ENSMUSG00000029765	Plxna4	001	chr6	32094565	32095925	-	-0.3	-5.2	3.7E-07	2.0E-03
ENSMUSG00000075003+ENSMUSG00000037876	Jmjd1c/Jmjd1c	041	chr10	66707622	66708166	+	0.7	5.1	4.2E-07	2.3E-03
ENSMUSG00000027799	Nbea	062	chr3	55986894	55987623	-	0.6	5.1	4.4E-07	2.3E-03
ENSMUSG00000023952	Gtpbp2	031	chr17	46302947	46303259	+	0.4	5.1	5.3E-07	2.7E-03
ENSMUSG00000042605	Atxn2	051	chr5	122261639	122261939	+	0.6	5.1	5.6E-07	2.8E-03
ENSMUSG00000003269	Cyth2	023	chr7	53068527	53069248	-	0.4	5.1	5.9E-07	2.9E-03
ENSMUSG00000022451	Twf1	001	chr15	94408382	94410096	-	0.2	5.2	6.1E-07	3.0E-03
ENSMUSG00000072647+ENSMUSG00000029454	Adam1a/Mapkapk5	002	chr5	121968622	121969392	-	0.4	5.1	6.9E-07	3.3E-03
ENSMUSG00000038664	Herc1	095	chr9	66348328	66348982	+	-0.6	-5.0	7.3E-07	3.4E-03
ENSMUSG00000030082	Sec61a1	014	chr6	88463896	88464200	-	0.8	5.1	7.6E-07	3.5E-03
ENSMUSG00000032855	Pkd1	017	chr17	24709563	24711715	+	0.5	5.0	8.4E-07	3.8E-03
ENSMUSG00000040929	Rfx3	001	chr19	27836211	27840930	-	-0.3	-5.0	9.7E-07	4.3E-03
ENSMUSG00000038762	Abcf1	037	chr17	36105913	36106178	-	1.4	4.9	1.3E-06	5.6E-03
ENSMUSG00000023952	Gtpbp2	044	chr17	46304840	46304970	+	0.4	4.9	1.3E-06	5.7E-03
ENSMUSG00000006676	Usp19	027	chr9	108403525	108404028	+	0.2	5.0	1.4E-06	5.8E-03
ENSMUSG00000078789+ENSMUSG00000038268	Dph1/Ovca2	001	chr11	74989444	74991144	-	0.3	5.0	1.4E-06	5.8E-03
ENSMUSG00000040896	Kcnd3	009	chr3	105468465	105469879	+	1.0	5.0	1.4E-06	5.9E-03
ENSMUSG00000030207	8430419L09Rik	017	chr6	135182873	135183273	+	-0.5	-4.9	1.7E-06	6.9E-03

ENSMUSG00000048148	Nwd1	031	chr8	75235492	75238645	+	-0.3	-4.4	1.6E-05	3.1E-02
ENSMUSG00000022514	Il1rap	028	chr16	26728315	26730203	+	-0.3	-4.4	1.7E-05	3.3E-02
ENSMUSG00000044783	Hjurp	023	chr1	90171673	90173793	-	0.5	4.4	1.7E-05	3.3E-02
ENSMUSG00000045482	Trrap	013	chr5	145545127	145545215	+	1.3	4.3	1.7E-05	3.3E-02
ENSMUSG00000005378	Wbscr22	030	chr5	135537215	135537339	-	0.9	4.4	1.7E-05	3.3E-02
ENSMUSG00000084896+ENSMUSG00000020883	Gm11632/Fbxl20	014	chr11	97956818	97958242	-	-0.6	-4.4	1.7E-05	3.3E-02
ENSMUSG00000052423	B4galt3	014	chr1	173201505	173201770	+	-0.8	-4.4	1.8E-05	3.4E-02
ENSMUSG00000031878	Nae1	009	chr8	107040890	107040949	-	1.0	4.3	1.8E-05	3.4E-02
ENSMUSG00000037996	Slc24a2	002	chr4	86629033	86637076	-	-0.2	-4.4	1.9E-05	3.5E-02
ENSMUSG00000028703	Lrrc41	005	chr4	115751487	115751587	+	1.1	4.4	1.9E-05	3.5E-02
ENSMUSG00000060206	Zfp462	005	chr4	55021187	55024237	+	0.4	4.4	1.9E-05	3.6E-02
ENSMUSG00000037017	Zscan21	015	chr5	138575442	138575442	+	2.3	4.4	2.0E-05	3.6E-02
ENSMUSG00000020716	Nf1	029	chr11	79258526	79258648	+	1.2	4.3	2.0E-05	3.6E-02
ENSMUSG00000031389+ENSMUSG00000031388+ ENSMUSG00000031391	Arhgap4/Naa10/L1cam	132	chrX	71163408	71164840	-	0.4	4.3	2.1E-05	3.7E-02
ENSMUSG00000032589	Bsn	010	chr9	108012745	108018857	-	0.4	4.4	2.1E-05	3.7E-02
ENSMUSG00000091471+ENSMUSG00000025204+ ENSMUSG00000051984	Gm20538/Ndufb8/Sec31b	015	chr19	44599966	44600144	-	-2.8	-4.3	2.1E-05	3.7E-02
ENSMUSG00000020894	Vamp2	013	chr11	68903553	68903678	+	0.5	4.4	2.1E-05	3.8E-02
ENSMUSG00000001763	Tspan33	001	chr6	29644222	29644233	+	1.9	4.4	2.3E-05	4.0E-02
ENSMUSG00000026596	Abl2	018	chr1	158572848	158579699	+	-0.3	-4.4	2.3E-05	4.0E-02
ENSMUSG00000050875	A730017C20Rik	012	chr18	59232072	59234318	+	-0.6	-4.4	2.3E-05	4.0E-02
ENSMUSG00000030082	Sec61a1	012	chr6	88462600	88463804	-	0.5	4.3	2.4E-05	4.2E-02
ENSMUSG00000040447	Spns2	007	chr11	72266618	72267055	-	0.5	4.3	2.4E-05	4.2E-02
ENSMUSG00000048078	Odz4	055	chr7	104057065	104059603	+	-0.2	-4.3	2.5E-05	4.2E-02
ENSMUSG00000057236	Rbbp4	016	chr4	129002068	129005831	-	0.4	4.3	2.5E-05	4.2E-02
ENSMUSG00000044308	Ubr3	054	chr2	69858185	69858507	+	-0.5	-4.3	2.5E-05	4.2E-02
ENSMUSG00000040209	Zfp704	001	chr3	9427011	9438898	-	-0.4	-4.4	2.6E-05	4.3E-02
ENSMUSG00000023026	Dip2b	023	chr15	100011740	100011867	+	0.6	4.2	2.8E-05	4.6E-02
ENSMUSG00000056602	Fry	027	chr5	151198318	151198442	+	0.5	4.2	2.8E-05	4.7E-02
ENSMUSG00000051306	Usp42	023	chr5	144483224	144483814	-	1.8	4.3	2.9E-05	4.7E-02
ENSMUSG00000035027	Map2k2	009	chr10	80581357	80581721	+	-0.9	-4.3	2.9E-05	4.7E-02
ENSMUSG00000007850	Hnrnph1	046	chr11	50199824	50199891	+	0.6	4.2	2.9E-05	4.7E-02
ENSMUSG00000029578	Wipi2	016	chr5	143140444	143140598	+	0.7	4.3	3.0E-05	4.8E-02
ENSMUSG00000027797	Dclk1	009	chr3	55270495	55275239	+	0.3	4.3	3.0E-05	4.8E-02
ENSMUSG00000028943	Espn	001	chr4	151494440	151494444	-	0.8	4.2	3.0E-05	4.8E-02

ENSMUSG00000017412	Cacnb4	001	chr2	52283845	52290269	-	0.3	4.8	3.0E-06	4.8E-03
ENSMUSG00000002984	Tomm40	008	chr7	20288492	20288617	-	0.6	4.8	3.2E-06	5.0E-03
ENSMUSG00000039759	Thap3	004	chr4	151359568	151359777	-	-0.5	-5.0	3.3E-06	5.2E-03
ENSMUSG00000038822	Hace1	010	chr10	45325391	45325393	+	2.1	4.7	3.4E-06	5.3E-03
ENSMUSG00000052373	Mpp3	012	chr11	101870999	101871520	-	0.7	4.7	3.4E-06	5.3E-03
ENSMUSG00000084708+ENSMUSG00000065862+ ENSMUSG00000059796	//Eif4a1	038	chr11	69484604	69484724	-	0.5	4.7	3.4E-06	5.3E-03
ENSMUSG00000025155	Dus1l	009	chr11	120651195	120651761	-	0.4	4.7	3.5E-06	5.3E-03
ENSMUSG00000036545	Adamts2	028	chr11	50617071	50621075	+	-0.8	-4.8	3.5E-06	5.3E-03
ENSMUSG00000038429	Usp5	025	chr6	124772861	124773037	-	0.7	4.7	3.5E-06	5.3E-03
ENSMUSG00000061887	Ssbp3	002	chr4	106584075	106584115	+	1.9	4.7	3.7E-06	5.6E-03
ENSMUSG00000084708+ENSMUSG00000065862+ ENSMUSG00000059796	//Eif4a1	039	chr11	69484725	69484952	-	0.5	4.7	3.8E-06	5.6E-03
ENSMUSG00000021087	Rtn1	003	chr12	73313276	73313276	-	-0.2	-4.8	3.8E-06	5.6E-03
ENSMUSG00000024012	Mtch1	029	chr17	29484412	29484704	-	0.3	4.7	3.8E-06	5.6E-03
ENSMUSG00000026885	Ttll11	024	chr2	35835145	35835286	-	0.6	4.7	3.9E-06	5.6E-03
ENSMUSG00000040896	Kcnd3	009	chr3	105468465	105469879	+	0.9	4.8	3.9E-06	5.6E-03
ENSMUSG00000040859	Bsdc1	017	chr4	129146293	129147418	+	0.7	4.7	3.9E-06	5.7E-03
ENSMUSG00000028063	Lmna	005	chr3	88286535	88286786	-	0.6	4.7	4.0E-06	5.7E-03
ENSMUSG00000053046+ENSMUSG00000092652	Brsk2/Mir3104	002	chr7	149135751	149135911	+	0.9	4.7	4.0E-06	5.7E-03
ENSMUSG00000032997+ENSMUSG00000026211	Chpf/Obsl1	060	chr1	75499791	75499941	-	-2.6	-4.7	4.0E-06	5.8E-03
ENSMUSG00000023952	Gtpbp2	035	chr17	46303816	46303936	+	0.7	4.7	4.2E-06	6.0E-03
ENSMUSG00000050989	Sepn1	019	chr4	134107852	134108081	-	1.2	4.8	4.3E-06	6.1E-03
ENSMUSG00000027223	Mapk8ip1	018	chr2	92241186	92241420	-	0.4	4.8	4.3E-06	6.1E-03
ENSMUSG00000031167	Rbm3	005	chrX	7719487	7719493	-	1.7	4.7	4.4E-06	6.1E-03
ENSMUSG00000047617	BC029214	024	chr2	25316142	25316174	-	-0.7	-4.7	4.4E-06	6.1E-03
ENSMUSG00000030447	Cyfip1	051	chr7	63185842	63185868	+	-0.8	-4.6	4.5E-06	6.3E-03
ENSMUSG00000013593	Ndufs2	015	chr1	173170159	173170186	-	0.8	4.7	4.7E-06	6.4E-03
ENSMUSG00000000441	Raf1	007	chr6	115570346	115571833	-	0.2	4.7	4.7E-06	6.5E-03
ENSMUSG00000027001+ENSMUSG00000026999	Dusp19/Nup35	032	chr2	80496235	80497345	+	-0.9	-4.7	5.0E-06	6.7E-03
ENSMUSG00000038822	Hace1	034	chr10	45420831	45429686	+	-0.3	-4.6	5.0E-06	6.7E-03
ENSMUSG00000073174+ENSMUSG00000040003	Magi2/Magi2	030	chr5	20208194	20208297	+	0.8	4.7	5.0E-06	6.7E-03
ENSMUSG00000025487	Psmd13	024	chr7	148076311	148076393	+	0.8	4.6	5.0E-06	6.7E-03
ENSMUSG00000036067	Slc2a6	009	chr2	26879856	26880104	-	-0.6	-4.7	5.1E-06	6.8E-03
ENSMUSG00000057236	Rbbp4	016	chr4	129002068	129005831	-	0.5	4.7	5.1E-06	6.8E-03
ENSMUSG00000031511	Arhgef7	028	chr8	11830238	11831492	+	0.3	4.7	5.1E-06	6.8E-03

***Highlights (for review)**

- A number of genes show alternative splicing during learning
- Homer1 isoform *Ania-3* is regulated by fear conditioning
- Differential isoform usage can vary with shock only, context only, or fear conditioning